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Chemotaxonomic Characterization of Microorganisms by Capillary Gas Chromatography-Mass Spectrometry

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Research is an interdisciplinary basic research program in biodetection using in mass spectrometry. Identification of specific pyrolytic chemical markers for microorganisms that are capable of identifying important biological threats is essential to the success of mass spectrometer-based systems for biodetection.

Results from the first model system to date have demonstrated clearly that decisive information for bacterial identification can be generated by analytical pyrolysis using chemical markers.

Derivation GC of bacterial samples may be simplified by the choice of selective chemical reactions and sample cleanup steps which remove many contaminating or interfering components.

A network of HP GC-MS data stations enables the interchange of data files between any two machines. A number of programs have been developed for data display and pattern (cont

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recognition of multivariate pyrolysis data. Currently the package performs data pretreatment and feature selection, principal component analysis, hierarchical single linkage clustering, and nonlinear mapping. The capabilities have been extended to include linear, quadratic, and stepwise discriminant analysis and other heuristic factor analysis approaches. Keywords; capillary gas chromatography; biodetection; mass spectrometry; (KT)

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5. **NAME OF INSTITUTION:** University of South Carolina

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7. LIST OF MANUSCRIPTS SUBMITTED OR PUBLISHED UNDER ARO SPONSORSHIP DURING THIS PERIOD, INCLUDING JOURNAL REFERENCES:

R. S. Whiton, S. L. Morgan, J. Gilbart, and A. Fox, "Modifications in the Alditol Acetate Method for the Analysis of Muramic Acid and Other Neutral and Amino Sugars by Capillary Gas Chromatography-Mass Spectrometry," J. Chromatography, 347, 109-120 (1985).

A. Fox, J. Gilbart, J. Harrison, G. Pararajasegaram, A. Wells, M. Hammer, C-H. Yang, Y. Ishikawa, R. S. Whiton, and S. L. Morgan, "Chemical and Immunological Studies on the in vivo and in vitro Degradation of Peptidoglycan and its Subunits and Cell Wall Complexes in Relation to Inflammatory Diseases", chapter in "Biological Properties of Peptidoglycan", H. P. Seidl and K. H. Schleifer, Eds., Walter de Gruyter & Co., Berlin (1986), p. 61-66.

S. L. Morgan, "GC-MS Characterization of Microorganisms and Other Biopolymers-- Pyrolysis and Data Analysis", chapter in "Mass Spectrometry Biological Detection Workshop", A. P. Snyder, Ed., 18-20 November 1985, Proceedings published by the Chemical Research, Development & Engineering Center, Aberdeen Proving Grounds, MD, May 1986, CRDEC-SP-86017.

J. Gilbart, A. Fox, R. S. Whiton, amd S. L. Morgan, "Rhamnose and Muramic Acid: Chemical Markers for Bacterial Cell Walls in Mammalian Tissue", Journal of Microbiological Methods, 5, 271-282 (1986).

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S. L. Morgan, A. Fox, and C. S. Smith, "Microorganism Differentiation by Analytical Pyrolysis GC-MS with Computer-assisted Pattern Recognition Techniques", chapter in "Proceedings of the 1986 U. S. Army Conference on Chemical Defense Research", Aberdeen, MD, 18-21 November 1986.

C. S. Smith, S. L. Morgan, C. Parks, A. Fox, and D. G. Pritchard, "Chemical Marker for the differentiation of Group A and Group B Streptococci by Pyrolysis Gas Chromatography- Mass Spectrometry," Analytical Chemistry, 59, 1410-1413 (1987).

S. L. Morgan, M. D. Walla, and J. C. Rogers, "Measuring and Displaying Chromatographic Similarity Using Cluster Analysis", Chromatography, 2(6), 12-20 (1987).

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A. Fox, J. Gilbart, B. Christensson, and S. L. Morgan, "Analysis of Carbohydrates for Profiling and Detection of Microorganisms", in press, Proceedings 4th Rapid Methods in Automation in Microbiology & Immunology, (1988).

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S. L. Morgan, A. Fox, and J. Gilbart, "Identification, structural characterization and trace detection of chemical markers for microorganisms by gas chromatography-mass spectrometry," J. Microbiol. Methods, in press (1988), submitted for a special issue on the "First International Symposium on the interface between Analytical Chemistry and Microbiology: Applications of Chromatography and Mass Spectrometry," held 3-5 June 1987 at the University of South Carolina, Columbia, SC.

R. S. Whiton and S. L. Morgan, "A Comparative Study on the Analytical Pyrolysis of Amino Acids," Journal of Analytical and Applied Pyrolysis, submitted (1987).

C. S. Smith, S. L. Morgan, C. D. Parks, and A. Fox, "Discrimination and Clustering of Streptococci by Pyrolysis Gas Chromatography-Mass Spectrometry", Journal of Analytical & Applied Pyrolysis, submitted (1988).

R. S. Whiton, S. L. Morgan, and F. L. Bayer, "Peptide Sequencing by Pyrolysis Gas Chromatography-Mass Spectrometry," in preparation for Journal of Analytical & Applied Pyrolysis (1986).

Miscellaneous articles about our research:

Mary D. Warner, Associate Editor, Analytical Chemistry, Editorial review (of research by S. L. Morgan and A. Fox), "Identification of microorganism by GC/MS", Analytical Chemistry, 58, 1310A-1316A (1986).

Michael J. Root, "Biotechnology breeds new diagnostic probes", review (of research by S. L. Morgan and A. Fox), Industrial Chemist, 38-42, December 1987.

8. SCIENTIFIC PERSONNEL SUPPORTED BY THIS PROJECT AND DEGREES AWARDED DURING THIS REPORTING PERIOD:

During the three years of this ARO contract, a number of graduate students, technicians, and two postdoctoral fellows have contributed to the progress of this research. Cheryl Parks (June 1985-present), a microbiology technician, has been responsible for the development of a culture collection and for preparation and characterization of cells and cell wall constituents. Sue Green (October 1985-May 1987), a technician in chemistry, has been involved in pyrolysis of bacteria. A computer programmer, Michael Abdalla (June 1985-May 1986, Chemistry) contributed to software development. Graduate students completing Ph.D. degrees in analytical chemistry on projects funded by ARO during this period include Robert S. Whiton (Ph.D., 12/85) and Cynthia S. Smith (Ph.D., 12/86). Current Ph.D. students contributing to our research in this project include Joseph Harrison (March 1985-present, Microbiology & Immunology) who has worked on amino acid derivatization, Kimio Ueda (January 1987-present, Chemistry) who is currently working on amino acid derivatization as well as CI MS identification of pyrolytic chemical markers, and James Rogers (October 1986-present, Chemistry) who is working on computer-assisted recognition of chemical markers in pyrograms. Dr. Bertil Christensson (visiting microbiologist from the University of Lund, Lund, Sweden, May 1987-present) who is working on negative ion CI and carbohydrate chemical markers. Dr. James Gilbart (March 1985-present), a postdoctoral fellow in Microbiology & Immunology, has been involved in both pyrolysis and derivatization studies and has overseen maintenance of GC-MS equipment. Several other students have finished Ph.D. degrees or are currently working in our laboratories on peripherally related projects. It should be clear that funding from our

current ARO contract has provided only a portion of the salary costs of these numerous individuals who have contributed to our progress.

9. BRIEF OUTLINE OF RESEARCH FINDINGS

Our research is an interdisciplinary basic research program in biodetection using in mass spectrometry. Identification of specific pyrolytic chemical markers for microorganisms that are capable of identifying important biological threats is essential to the success of mass spectrometer-based systems for biodetection. The identification of relevant chemical markers is typically achieved by research efforts in four separate stages: experimental design to select groups of microorganisms, cell fractions, and model compounds that have the potential to permit chemical markers to be identified; adequate biological and chemical characterization of microbiological samples prior to pyrolysis; pyrolysis GC-MS analysis of selected microbial samples; and, automated identification of chemical markers using pattern recognition methods. Our studies are prototypes for the pyrolysis MS-based identification of bacteria and, more importantly, are designed to identify specific chemical markers that have practical utility for biodetection.

The ideal outcome of these investigations will provide information that will reproducibly permit any laboratory (including pyrolysis systems in the field) to identify the selected microorganisms. This identification will be based on known structural differences and thus will be independent of the particular instrumentation. Furthermore, chemical components of the microorganisms will be identified which do not vary significantly with the manner in which the agent has been grown, prepared, packaged, and delivered to the battlefield. Although GC-MS and MS/MS generate different information and have different limitations, results achieved by pyrolysis GC-MS should be reproducible by MS/MS. Correlation between pyrolysis GC-MS and MS-MS results must be established experimentally, however, and one collaborative study has been initiated with Dr. Peter Snyder (CRDEC), involving the MS/MS identification of a chemical marker for group B streptococci previously found by pyrolysis GC-MS.

The period of our initial funding from the Army Research Office began 1 March 1985 and ended 28 February 1988. During this three-year period our laboratories were jointly funded a total of $180,000. Instrumentation acquired in the first year included a Hewlett-Packard 5970 Mass Selective Detector which was purchased with $15,000 from the ARO contract, $30,000 matching funds from the University of South Carolina, and the donation of a HP 5880 GC (ca. $20,000). Two additional GC-MS systems (one in each laboratory) had been previously funded by grants from NIH (1979) and NSF (1984). Last year we received a Department of Defense Instrumentation Grant of $97,600 which was matched by $103,000 from the University of South Carolina. This instrument grant enabled us to acquire a Hewlett-Packard 5988 GC-MS system and to upgrade existing GC-MS equipment to a high level of compatibility.

A. Pyrolysis Studies

Rhamnose-containing polysaccharides are major components of the cell walls of streptococci and their serological differentiation is based on indirectly detecting differences in carbohydrate composition (Lancefield grouping). The group B-specific polysaccharide consists of a backbone of rhamnose and glucitol phosphate residues with trisaccharide sidechains composed of rhamnose, galactose, and N-acetylglucosamine covalently bound to the peptidoglycan lattice of the cell wall (45). Derivatization GC analysis of streptococci had previously shown glucitol to be present only in Group B strains (15).

In recent work, we demonstrated Group B and A streptococci can be differentiated by the formation of a unique carbohydrate pyrolysis product generated from the glucitol moiety. We have further used pattern recognition to differentiate several Lancefield groups of streptococci from one another including groups A, B, C, F, and G. This marker may be identified by selected or reconstructed ion monitoring GC-MS using the prominent ion at m/z 86. We have not yet identified the precise chemical structure of this pyrolysis product. The highest mass ion present in the EI mass spectrum is at m/z 103; if this mass represents the molecular ion, the pyrolysis product could be a 4-carbon anhydroalditol; ions at m/z 86 and m/z 69 could represent the successive loss of hydroxyl groups. The mass of 103 is not consistent with an expected mass of 102 for the 4-carbon anhydroalditol.

Further work, including a comparison of current information on carbohydrate pyrolysis products is necessary to resolve the structure of this unique pyrolysis product. We are currently using our new Hewlett-Packard 5988 GC-MS system with chemical ionization following analytical pyrolysis to obtain more structural information and a better estimate of the molecular ion for this pyrolysis product; CI-MS may also provide additional discrimination among groups not available using EI-MS. We have also provided samples to Dr. Peter Snyder (CRDEC) and Dr. Rick Yost (U. Florida) to see if this unique chemical marker can also be identified by pyrolysis atmospheric pressure chemical ionization MS-MS; ongoing discussions on the design and interpretation of their results may result in useful comparative information.

Results from this first model system to date have demonstrated clearly that decisive information for bacterial identification can be generated by analytical pyrolysis using chemical markers.

B. Characterization of microorganisms and microbial constituents

Derivatization GC of bacterial samples may be simplified by the choice of selective chemical reactions and sample cleanup steps which remove many contaminating or interfering components. Details of the procedures may be found in our published papers .

Carbohydrate profiling on samples is done by the alditol acetate procedure and GC-MS. A chapter describing the background and details of our modified alditol acetate procedure for carbohydrate analysis will be published shortly in a book "Carbohydrate Analysis by Gas Chromatography and Mass Spectrometry," CRC, 1988.

Amino acid content of bacteria is be assayed by the GC-MS analysis of the N-heptafluorobutyryl butyl esters. Under the auspices of an NSF travel award, Dr. Fox has collaborated with Lennart Larsson and Goran Odham (University of Lund, Sweden) who have pioneered the se of amino acids as chemical markers for bacteria.

Fatty acid analysis by GC is considerably less complicated than sugar or amino acid analysis since only one functional group requires derivatization. Dr. James Gilbart, a postdoctoral fellow who has worked in our laboratories since January 1985 specialized in fatty acid GC analysis in his doctoral research.

C. Computer-assisted data handling

Our network of HP GC-MS data stations enables the interchange of data files between any two machines. Complete GC-MS data files including raw data, total ion abundance data, selected ion data, or integrated area reports can be downloaded to a Zenith Z-248 PC. This machine is an IBM-PC-AT clone running at 10 Mhz with 1.5 M of internal memory

and a 40 M hard disk drive. We have implemented software that permits us complete access to all forms of data generated by the GC-MS instrumentation. Acquired GC-MS data, consisting of peak intensities for selected ions as a function of retention time or mass spectra as a function of time, are stored in ASCII files appropriately configured for further data analysis. Programs are written in one of three different languages (Microsoft FORTRAN, Microsoft QUICKBASIC, and Turbo Pascal) depending on the application. In addition to HP graphics plotters directly attached to the GC-MS data stations, we have two Hewlett-Packard 7440 ColorPro graphics plotters interfaced to the off-line PC's for publication quality graphic displays of GC-MS data and pattern recognition results. HP terminal emulation software and a Hayes modems or RS-232 cables are employed to connect the off-line PC's or the HP data stations for uploading data to the College VAX 11/780. Networking the instruments unifies our data treatment and enables access to superior computing capability, graphics, and mass storage.

Preliminary feature selection and data transformation is carried out on the PC-AT's. Pattern recognition software can perform exploratory data analysis or pattern classification on this level. In our previous pyrolysis GC-MS studies, the number of chemical components (pyrolysates) to be analyzed has been limited to simplify data handling and interpretation. There is, however, no reason to ignore a large part of the information that is available in a high resolution capillary GC-MS pyrogram-- as long as a means of efficiently processing the data is available. Data handling techniques that we have previously employed have been off-line and conducted on a mainframe computer far removed in time and space from the pyrolysis experiment. In a recent study, we demonstrated an automated approach to data handling in analytical pyrolysis by transferring files from the HP GC-MS data stations to our college VAX where the data was treated using SAS, a commercially available pattern recognition software.

We have developed number of programs for data display and pattern recognition of multivariate pyrolysis data. This software is presently running in compiled Microsoft FORTRAN or QUICKBASIC on an IBM PC. Currently the package performs data pretreatment and feature selection, principal component analysis, hierarchical single linkage clustering, and nonlinear mapping. We have expanded the capabilities to include linear, quadratic, and stepwise discriminant analysis and other heuristic factor analysis approaches.